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Does β-Carotene Explain Why Reduced Cancer Risk Is Associated with Vegetable and Fruit Intake?[1]

Regina G. Ziegler,[2] Amy F. Subar, Neal E. Craft, Giske Ursin, Blossom H. Patterson, and Barry I. Graubard

Environmental Epidemiology Branch, Epidemiology and Biostatistics Program, Division of Cancer Etiology [R. G. Z.] and Applied Research Branch, Surveillance Program, Division of Cancer Prevention and Control [A. F. S.], National Cancer Institute, Bethesda, Maryland 20892; Organic Analytical Research Division, Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899 [N. E. C.]; Department of Epidemiology, School of Public Health, University of California, Los Angeles, California 90024 [G. U.]; Biometry Branch, Division of Cancer Prevention and Control, National Cancer Institute, Bethesda, Maryland 20892 [B. H. P., B. I. G.]

Abstract

Increased intake of vegetables, fruits, and carotenoids and elevated blood levels of β-carotene are consistently associated with reduced risk of lung cancer in epidemiologic studies. Epidemiologic research also suggests that carotenoids may reduce the risk of other cancers, although the evidence is less extensive and consistent. The simplest explanation is that β-carotene is protective. However, the possible roles of other carotenoids, other constituents of vegetables and fruits, and associated dietary patterns have not been adequately explored. To evaluate these alternative hypotheses, we are undertaking three lines of research. (*a*) With dietary data from the 1987 National Health Interview Survey and the 1982–1984 Epidemiologic Follow-up of the first National Health and Nutrition Examination Study, we have determined which food groups and nutrients are highly correlated with vegetable and fruit intake. (*b*) We have developed and characterized a liquid chromatography method for optimal recovery and resolution of the common carotenoids in blood, specifically lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene. (*c*) In a population-based case-control study of lung cancer in white men in New Jersey, we are assessing whether estimates of the intake of the individual carotenoids might produce stronger inverse associations than estimates of provitamin A carotenoids based on current food composition tables.

The hypothesis that β-carotene can reduce the risk of cancer has been proposed relatively recently. Previously, attention focused on vitamin A because of its recognized role in normal cell differentiation and because high doses of retinoids (vitamin A analogues) limited carcinogenesis in animal experiments. In the 1980s interest in β-carotene escalated for several reasons. First, β-carotene is the most abundant and the most efficiently converted of the provitamin A carotenoids (vitamin A precursors) in vegetables and fruits. Second, a protective role for β-carotene explained the early epidemiologic evidence that increased vegetable and fruit intake reduced cancer risk. Third, serum levels of β-carotene are responsive to dietary intake, unlike serum levels of vitamin A, which are maintained within a narrow range in well-nourished populations by vitamin A stored in the liver. Finally, a plausible mechanism was postulated for β-carotene, as an antioxidant, that did not require its conversion to vitamin A.

Prospective Studies of Carotenoid Intake and Cancer

Before questioning whether the β-carotene hypothesis really explains the epidemiologic findings, it is necessary to review the evidence. First, prospective studies and then a limited number of retrospective studies will be considered. In a prospective study, dietary information and/or blood samples are collected from a group of nondiseased people, and this cohort is followed over time. When a sufficient number of cancer diagnoses or deaths have occurred, the data collected earlier are compared for the cases and all the noncases in the cohort or for the cases and matched controls selected from the cohort. Thus exposure, whether measured by dietary intake or blood nutrient levels, is ascertained before clinical disease.

Seven prospective studies of carotenoid intake and cancer were published (1–8), with the earliest appearing in 1979 (1); locations included Japan (1, 2), Norway (4), the Netherlands (8), and the United States (3, 5–7). The exposure most frequently evaluated was vegetable and fruit intake. Only three studies (3, 7, 8) actually assessed carotenoid intake by including in the interview most of the major sources of carotenoids in the diet and developing a quantitative index by weighting the frequencies of consumption of these foods by their measured carotenoid content, based on food composition tables.

Not all of these studies looked at the same cancers; only two (3, 7) systematically investigated the most prevalent cancers in their cohort. Three (1, 2, 5, 7) of the five studies (1, 2, 3, 5, 7, 8) that analyzed all cancers combined found a reduced risk with increased intake of vegetables and fruit and/or carotenoids. Five (1–4, 6, 8) of six studies (1–4, 6–8) found a reduced risk of lung cancer with increased intake. A reduced risk of breast (7), cervical (1, 2), stomach (1, 2), and oral-pharyngeal (3) cancer was noted in the single study that investigated each cancer. However, risk of bladder cancer was decreased in only one (7) of two (3, 7) studies; and no protective effect was seen for the intake of vegetables and fruit or of carotenoids for prostate cancer in three studies (1–3, 7), for colon cancer in two studies (3, 7), or for skin cancer in one study (3).

Thus in these prospective studies of diet and cancer, high levels of vegetable and fruit intake are consistently associated with a reduced risk of lung and possibly other cancers. However, the protective factor is difficult to identify. Only one study systematically investigated the relationship of all the common nutrients to the risk of lung cancer; carotenoid intake alone was significantly associated with reduced risk (3). The roles of constituents of vegetables and fruits other than nutrients and of individual carotenoids were not evaluated in any of these studies. In addition, the one study that compared the impact of vegetable, fruit, and carotenoid intake found that fruit was the most predictive of reduced lung cancer risk (8). Dietary retinol (preformed vitamin A) was not protective in three (3, 7, 8) of four lung cancer analyses (3, 4, 7, 8), which suggests that β-carotene does not first have to be metabolized into retinol to be effective.

Prospective Studies of Blood Carotenoid Levels and Cancer

Prospective studies have looked not only at vegetable and fruit and carotenoid intake but also at carotenoid concentra-

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[2] To whom requests for reprints should be addressed, at Environmental Epidemiology Branch, National Cancer Institute, Executive Plaza North 443, Bethesda, MD 20892.

tions in serum or plasma before cancer onset. Seven such studies were published (9–20), with locations including the continental United States (9, 13–17, 19), Hawaii (12), England (18), Switzerland (10, 11), and Finland (20). In the 7 years since the first of these studies was published, evaluation of exposure has become more sophisticated. The earliest study measured total carotenoids by colorimetry (9); later studies used LC[3] to separate and measure β-carotene (10–14, 18–20). Recently blood levels of other individual carotenoids, such as lycopene, were also quantitated (15–17).

All of these studies systematically tested for associations with each of the common cancers in their cohorts. A reduction in risk of all cancer with high blood β-carotene levels was noted in two (18, 20) of the three studies (18–20) that analyzed all sites combined. A reduction in lung cancer risk was associated with elevated blood β-carotene levels in five (10–13, 18, 19) of six studies (10–13, 18–20); the inverse associations were statistically significant in four of these studies (10–13, 18); trends were seen in all five. Also, a reduced risk of stomach cancer was consistently associated with high β-carotene levels in three (10–12, 18) of four studies (10–12, 18, 20). However, for bladder cancer there was no apparent effect of high β-carotene in two (12, 16) of four studies (12, 16, 18, 20) or for colon cancer in three (10, 11, 14, 19) of five studies (10–12, 14, 18, 19). The three analyses of prostate cancer indicated no protection associated with high β-carotene (17, 20) or total carotenoid (9) concentrations.

Although the simplest explanation of these prospective studies is that β-carotene can reduce the risk of lung and stomach cancer, other carotenoids and other constituents of vegetables and fruits were not evaluated in a systematic fashion; elevated blood β-carotene levels may simply indicate increased consumption of vegetables and fruits. It is provocative that in one cohort, subjects with high serum lycopene levels had significantly reduced risks of pancreas and bladder cancer and a nonsignificantly reduced risk of prostate cancer, but no comparable β-carotene effects were seen (15–17). Serum β-carotene levels were inversely associated with risk of lung cancer in this cohort, but lycopene was not measured in these blood samples (13). Like β-carotene, lycopene is an antioxidant and may be more effective in certain tissues (21); however, it may also simply be an indicator of vegetable and fruit intake. In the one study that assayed for micronutrients shortly after blood collection, plasma vitamin C was more strongly associated with reduced risk of stomach cancer than was β-carotene (10, 11), which is consistent with other evidence that vitamin C plays a role in the etiology of stomach cancer (22). However, only plasma β-carotene, not vitamin C, was inversely associated with lung cancer risk in this study. Vitamin C, like β-carotene, is concentrated in vegetables and fruits; in general, it has not been evaluated adequately in studies of nutrition and cancer because of its lability in blood. However, methods are now available for stabilizing vitamin C for long-term storage (23). In six (9, 10–13, 19, 24) of the seven analyses of lung cancer outcomes in these cohorts (9, 10–13, 19, 20, 24), strong and/or consistent associations for retinol (vitamin A) similar to those for β-carotene were not seen, suggesting that β-carotene need not first be converted to retinol to be active.

[3] The abbreviations used are: LC, liquid chromatography; NCI, National Cancer Institute; NHIS, National Health Interview Survey; NIST, National Institute of Standards and Technology.

Retrospective Studies of Carotenoid Intake and Lung Cancer

A number of retrospective studies of carotenoids and specific cancers also were conducted. In a retrospective case-control study, patients with a particular cancer are identified and comparable control subjects are selected. Information about usual diet before signs of disease, blood samples, or both are then collected and compared for the cases and controls. Although there is a possibility for bias when cancer patients recall usual dietary patterns, no clear evidence for bias exists in a number of well-conducted studies. Lung has been the site studied most intensively in retrospective studies because of the prevalence of this cancer and the increasing evidence from epidemiologic studies that diet may be involved in its etiology. Fifteen retrospective studies of carotenoid intake and lung cancer have appeared since 1977 (25–40). Investigations were conducted in the continental United States (27–30, 32, 33, 35, 37), Hawaii (26, 36), Canada (39), Italy (31, 34), France (40), Australia (38), and Singapore (25). Retrospective studies of blood carotenoid levels and lung cancer will not be considered here. The severity of cancer and its treatment suggest that appetite and metabolism would be altered and would complicate the interpretation of nutrient levels in blood drawn after diagnosis.

Twelve (26–30, 32–37, 39, 40) of these 15 lung cancer studies formed a quantitative index of carotenoid intake; the others evaluated vegetable and fruit intake. Fourteen (25–37, 39, 40) of the 15 studies demonstrated a decreased risk of lung cancer with increased intake of carotenoids or green or yellow-orange vegetables. In all 14 analyses, inverse associations or trends were statistically significant. This consistency among epidemiologic studies is remarkable.

Risk of lung cancer was inversely associated with dietary vitamin C in four of these studies (26, 32, 35, 36) and with dietary fiber in two (32, 36). However, the associations were generally weaker than those with carotenoid intake. Intake of retinol (preformed vitamin A) seemed unrelated to risk in 9 (26–30, 32, 33, 36, 38, 39) of 12 (26–30, 32–36, 38–40) studies, once again suggesting that β-carotene need not first be converted to retinol to be protective. Only four of the studies compared the influence of vegetables and fruits with estimated carotenoid intake. Three found that vegetable intake was more predictive of reduced lung cancer risk than was the carotenoid estimate (27, 28, 36, 39), whereas the fourth found that fruit intake was more predictive (35). As will be discussed later, these results suggest that β-carotene content may not explain why vegetables and fruits are protective, but interpretation of these results is complicated by the lack of reliable food composition data for individual carotenoids, such as β-carotene.

Seven of these retrospective studies of dietary carotenoids and lung cancer used general population (26–29, 36, 39) or neighborhood (30, 32) controls, not hospital controls. Thus the dietary patterns for the controls are representative of healthy, typical populations. When the controls in these studies were stratified into quartiles or tertiles on the basis of vegetable and fruit and carotenoid intake, smoking-adjusted relative risks of lung cancer ranged from 1.4 to 2.2 in low consumers relative to high consumers. From a public health perspective, this implies that adoption of the levels of vegetable and fruit and carotenoid intake characteristic of the upper 30% of a typical community might be sufficient for a noticeable (29–55%) reduction in lung cancer risk in the lower 30% of vegetable and fruit consumers. Were all members of the community to adopt

the levels of vegetable and fruit consumption characteristic of the upper 30%, the risk of lung cancer in the community might be reduced by 15–31%.

On the basis of these and other findings, the National Cancer Institute recommends eating five or more servings of vegetables and fruits a day (41). Optimal intake of vegetables and fruits has not yet been determined scientifically. Nonetheless, approximately 9% of Americans presently consume five or more servings of vegetables and fruits a day (42). Thus this level of consumption typifies the upper range of vegetable and fruit intake in the United States and is feasible as well as prudent.

Retrospective Studies of Carotenoid Intake and Other Cancers

Retrospective studies indicate that vegetable and fruit intake may also reduce the risk of other cancers. Because of fewer studies and/or less consistency among studies, the epidemiologic evidence for a role for carotenoids is at present less persuasive than for lung cancer. Other cancers for which there is suggestive evidence from retrospective studies that vegetables, fruits, and possibly carotenoids are protective include cancer of the mouth, pharynx, larynx, esophagus, stomach, colon, rectum, bladder, cervix, and breast.

Alternatives to the β-Carotene Hypothesis

To summarize, a reduced risk of lung cancer is consistently observed with increased dietary intake of vegetables and fruits and carotenoids in prospective and retrospective studies. In addition, a reduced risk of lung cancer is consistently associated with high blood levels of β-carotene in prospective studies. The simplest explanation is that β-carotene is protective. However, alternative hypotheses have not been adequately explored. Other plausible explanations include (*a*) other carotenoids, (*b*) other constituents of vegetables and fruits, and (*c*) dietary patterns tightly correlated with frequent vegetable and fruit consumption.

Dietary Patterns Associated with Vegetable and Fruit Consumption

To help evaluate these alternative hypotheses, we are undertaking three lines of research. First, we are attempting to identify the dietary patterns associated with high vegetable and fruit consumption. From the perspective of cancer etiology, this effort may suggest alternatives to the β-carotene hypothesis that need to be evaluated in epidemiologic and experimental studies. In addition, from a public health perspective, it will point to correlated dietary patterns with nutritional and health consequences that must be considered when NCI advocates a diet rich in vegetables and fruits (41).

We are currently using two nationally representative dietary surveys to identify the nutrients, food groups, and food preparation practices associated with high vegetable and fruit intake. We are using the 60-item food frequency interview administered to 20,000 adults, aged 19 to 90 years, in the 1987 NHIS (43) and the 115-item food frequency interview administered to 10,000 adults, aged 32 to 75 years, in the 1982–1984 Epidemiologic Follow-up of the First National Health and Nutrition Examination Study (44, 45). Both interviews focused on diet during the past year. Preliminary results from the two data sets were similar, and only the NHIS findings are presented here.

Table 1 *Correlation between vegetable and fruit intake and nutrient intake in the 1987 NHIS*[a]

Macronutrients	r[b]	Micronutrients	r[b]
Calories	0.17	Vitamin A	0.45
% calories from fat	−0.25	Carotenoids	0.60
% calories from carbohydrates	0.27	Vitamin C	0.79
% calories from protein	0.06	Thiamin	0.38
Saturated fatty acids	0.007	Riboflavin	0.24
Linoleic acid	0.05	Folate	0.61
Cholesterol	0.06	Calcium	0.24
Dietary fiber	0.70	Iron	0.33

[a] Before correlation coefficients were computed, individual intake was expressed as a percentile, based on distribution of intake in NHIS participants of same sex and age group (18–34, 35–49, 50–64, 65+ years).
[b] Pearson's correlation coefficient.

Table 2 *Correlation between vegetable and fruit intake and food group consumption in the 1987 NHIS*[a]

Food group	r[b]	Food group	r[b]
Vegetables	0.81	Dairy products	0.12
Dark green vegetables	0.46	Starches	0.16
Yellow-orange vegetables	0.50	Cereals	0.23
Fruits	0.71	Breads	0.04
Citrus fruits	0.67	Desserts	−0.001
Beef/pork	−0.01	Sodas	−0.16
Poultry/fish	0.21	Alcohol	0.02
Processed meats	−0.08		

[a] Before correlation coefficients were computed, individual intake was expressed as a percentile, based on distribution of intake in NHIS participants of same sex and age group.
[b] Pearson's correlation coefficient.

Table 3 *Categorization of 1987 NHIS participants by intake of vegetables and fruits and of vitamin C*

	Vegetables and fruits[a]			
Vitamin C[a]	Lowest quartile	Quartile 2	Quartile 3	Highest quartile
Lowest quartile	17[b]	5.7	1.6	0.3
Quartile 2	5.9	11	6.7	1.8
Quartile 3	1.4	6.6	10	6.5
Highest quartile	0.2	1.7	6.2	17

[a] Individual intake was expressed as a percentile, based on distribution of intake in NHIS participants of same sex and age group.
[b] Numbers refer to percentage of NHIS participants in category.

When nutrient intake was evaluated (Table 1), vitamin C, dietary fiber, folate, and carotenoids were highly associated with servings per week of vegetables and fruits, with the correlation coefficients ranging from 0.8 to 0.6. Percentage of calories from fat was inversely correlated with vegetable and fruit intake ($r = -0.25$), although absolute intake of saturated fatty acids, polyunsaturated fatty acids, and cholesterol seemed unrelated. When food group consumption was considered (Table 2), certain vegetable and fruit subgroups, such as dark green vegetables and yellow-orange vegetables, were only moderately correlated with total vegetable and fruit intake ($r = 0.5$). Red meat (beef and pork) intake was not associated; however, poultry and fish intake, which in a recent study was related to a reduced risk of colon cancer (46), was weakly positively associated. Intakes of many major food groups, such as dairy products, starches, breads, desserts, and alcohol, seemed unrelated to vegetable and fruit intake.

Effects of exposures that were highly correlated, such as vegetable and fruit intake and vitamin C intake ($r = 0.8$), would be difficult to evaluate separately in an epidemiologic study of typical Americans. As shown in Table 3, 55% of the population ranked in the same quartile for both these exposures (Q1/Q1 + Q2/Q2 + Q3/Q3 + Q4/Q4), whereas only 7% differed by more than one quartile (Q1/Q3 + Q1/Q4 + Q2/Q4 + Q3/Q1

Table 4 *Categorization of 1987 NHIS participants by vegetable and fruit intake and percentage of calories from fat*

Percentage of calories from fat	Vegetables and fruits[a]			
	Lowest quartile	Quartile 2	Quartile 3	Highest quartile
Lowest quartile	9.0[b]	7.0	5.7	3.6
Quartile 2	6.5	6.7	6.5	5.5
Quartile 3	5.2	6.3	6.7	7.0
Highest quartile	4.0	4.8	6.1	9.4

[a] Individual intake was expressed as a percentile, based on distribution of intake in NHIS participants of same sex and age group.
[b] Numbers refer to percentage of NHIS participants in category.

Table 5 *Median intake of nutrients and food groups by quartile of vegetable and fruit intake in the 1987 NHIS*

Nutrient or food group	Vegetable and fruit intake[a]			
	Lowest quartile	Quartile 2	Quartile 3	Highest quartile
Vitamin C, mg	52	90	123	184
Carotenoids, μg	1148	1832	2513	3913
Dietary fiber, g	5.9	8.0	10.0	13.4
Folate, μg	159	205	247	313
Dark green vegetables, servings/week	0.47	0.93	1.23	2.00
Yellow-orange vegetables, servings/week	0.47	1.00	1.23	2.23

[a] Individual intake was expressed as a percentile, based on distribution of intake in NHIS participants of same sex and age group.

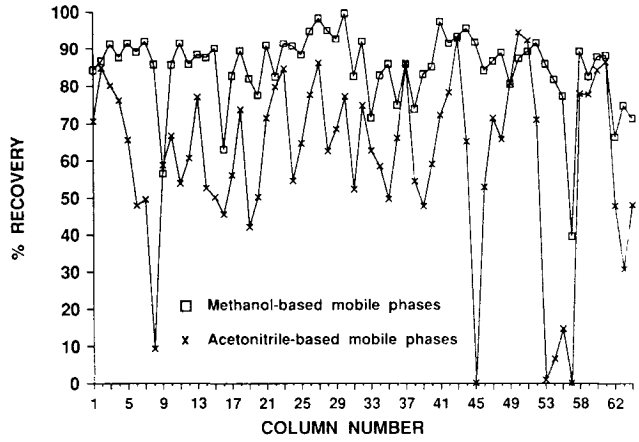

Fig. 1. Total recovery of a mixture of seven carotenoids (lutein, zeaxanthin, β-cryptoxanthin, echinenone, lycopene, α-carotene, and β-carotene) from 64 commercially available reversed-phase LC columns using methanol- and acetonitrile-based mobile phases.

+ Q4/Q1 + Q4/Q2). However, vegetable and fruit intake and percentage of calories from fat were only moderately correlated ($r = -0.25$), and their effects should be separable in a sufficiently large epidemiologic study. As demonstrated in Table 4, only 32% of the population was ranked in the equivalent quartiles for both exposures (25% would be expected if the exposures were statistically independent), and almost as many, 29%, differed by more than one quartile.

Table 5 demonstrates the striking gradient in absolute intake of selected nutrients and food groups across quartiles of vegetable and fruit consumption and suggests the potential public health impact of programs targeting vegetables and fruits. For vitamin C, carotenoids, dietary fiber, folate, dark green vegetables, and yellow-orange vegetables, the median absolute intake in the highest quartile of vegetable and fruit consumption was two to four times the median intake in the lowest quartile. Thus it is prudent to investigate whether the dietary patterns associated with high vegetable and fruit intake suggest any nutritional or medical concerns, especially in vulnerable subgroups.

Development of an LC Method with Improved Separation and Recovery of Individual Carotenoids

Our second line of research concerns the consistently observed increase in subsequent incidence of lung and stomach cancer for people with low serum or plasma β-carotene levels. Although these observations point to a protective role for β-carotene, low blood β-carotene levels may simply be an indicator of decreased intake of all carotenoids and of vegetables and fruits in general. Other carotenoids and constituents of vegetables and fruits need to be measured in these studies.

More than 600 carotenoids, all yellow to red (47), exist in biological materials; 5 to 10 separable, structurally distinct carotenoids are typically identified in serum or plasma collected from U.S. populations (48). β-Carotene is the most abundant and the most efficiently converted, of the provitamin A carotenoids (vitamin A precursors) in vegetables and fruits. However, lycopene is often the carotenoid at highest concentrations in serum in the United States (48). Many of the carotenoids, not just β-carotene, are effective antioxidants; carotenoids probably evolved to protect plants from reactive oxygen species generated in photosynthesis.

As we began to evaluate liquid chromatography methods for separating the major individual carotenoids in human serum and plasma, it became apparent that there was very little information published on recovery of the individual carotenoids. One reason was the lack of availability of pure reference materials for carotenoids other than β-carotene. Another was that cancer research was focused on β-carotene. Poor recovery during measurement of a carotenoid could lead to an imprecise estimate of exposure and thus obscure an association. In addition, spurious associations could be generated by differential recovery between cases and controls.

The Environmental Epidemiology Branch of NCI, in collaboration with the NIST, decided to develop an LC method for measuring individual carotenoids in human serum and plasma that would give both excellent resolution and recovery. In addition, the method had to be reproducible and practical so that it could be used by a variety of laboratories on the large numbers of samples collected in epidemiologic studies. Research indicated that a polymeric octadecylsilane stationary phase gave better resolution than a monomeric octadecylsilane phase, narrow pore packings were more reproducible than wide pore, and methanol or buffered acetonitrile mobile phases and biocompatible fruits produced the highest recoveries (49). Switching from an unbuffered acetonitrile-based mobile phase to a methanol-based phase gave the most striking increase in recovery. Fig. 1 demonstrates the improved recovery of carotenoids obtained on 58 of 64 LC columns by using a methanolic mobile phase.

Percentage recovery of the common serum carotenoids (lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene), with the new NIST-NCI LC method and with three accepted LC methods that have been used in epidemiologic studies, are presented in Table 6. The columns and mobile phases used in replicating these three LC methods were those published in the literature. Recovery was measured by flow injection analysis (SO). The NIST-NCI method gives 93–99% recovery of each of the six carotenoids. Recovery drops to 70% or less for lycopene and to 80% or less for at least one additional

Table 6 *Percentage recovery of individual carotenoids with new NIST-NCI LC method and three LC methods previously used in epidemiologic studies*

Method	Lutein[a]	Zeaxanthin[a]	β-Cryptoxanthin	Lycopene	α-Carotene	β-Carotene
NIST/NCI	95	94	93	97	99	99
A	80	75	82	68	89	91
B	99	98	85	70	77	84
C	99	91	96	101	94	91

[a] Lutein and zeaxanthin coelute in all methods except NIST-NCI.

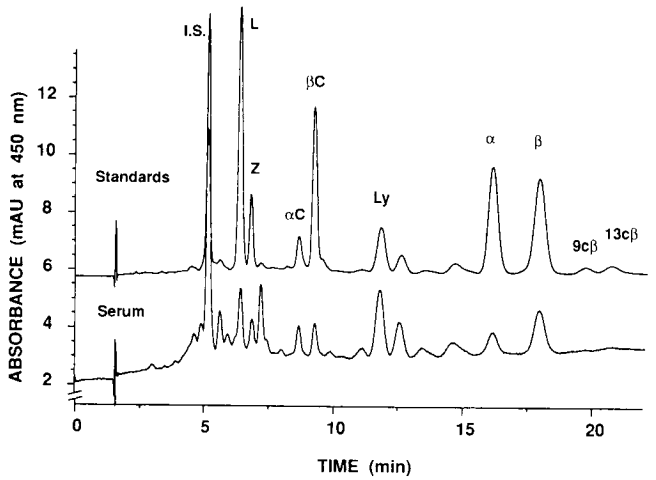

Fig. 2. Resolution of serum carotenoids with the NIST-NCI LC method. *Upper tracing*, mixture of the six most common serum carotenoids: lutein (*L*); zeaxanthin (*Z*); β-cryptoxanthin (*βC*); lycopene (*Ly*); α-carotene (*α*); and β-carotene (*β*); also seen are α-cryptoxanthin (*αC*), several unlabeled geometric isomers of lycopene, 9-*cis*-β-carotene (*9cβ*), and 13-*cis*-β-carotene (*13cβ*). *Lower tracing*, extract of human serum. The internal standard (*I.S.*) is β-apo-8′-carotenal. LC conditions: Bakerbond C_{18} column, gradient elution at 2.0 ml/min, 27°C, detection at 450 nm. Solvent A = 90% acetonitrile/10% ethyl acetate; solvent B = 90% methanol/10% ethyl acetate containing 100 mM ammonium acetate; gradient = 100% A for 2 min, linear to 50% A/50% B over 4 min, linear to 100% B over 14 min.

Table 7 *Smoking-adjusted relative risks of lung cancer in current and recent cigarette smokers: New Jersey white males*[a,b]

Nutrient or food group	Level of consumption: Upper 25%	Middle 50%	Lower 25%	*p* for trend
Retinol	1.0	1.1	1.0	0.48
Carotenoids	1.0	1.5	1.7	0.02
Vitamin A	1.0	1.2	1.2	0.26
Dairy products	1.0	0.8	0.9	0.26
Vegetables and fruit	1.0	1.7	1.8	0.005
Fruit	1.0	1.4	1.2	0.28
Vegetables	1.0	1.3	1.7	0.004
Dark green vegetables	1.0	1.4	1.8	0.002
Yellow-orange vegetables	1.0	1.6	2.2	<0.001

[a] Included are 524 cases and 354 controls.
[b] Adapted from Ziegler *et al.* (28).

carotenoid with methods A and B. Method C gives quite good recovery of all six carotenoids, but its resolution of individual carotenoids is limited. Of the four methods, only the NIST-NCI method can resolve the structural isomers lutein and zeaxanthin.

The ability of the NIST-NCI method to separate individual serum carotenoids is demonstrated in Fig. 2. Not only are structural isomers that frequently coelute (α-cryptoxanthin and β-cryptoxanthin, and lutein and zeaxanthin) resolved but geometric isomers are also separated. The two small peaks trailing the all-*trans* β-carotene peak are the 9-*cis* and 13-*cis* isomers, which together comprise approximately 10% of total serum β-carotene. The two peaks after all-*trans* lycopene and the peak before it contain its geometric isomers. Analysis time for this separation is 20 min; total run time including reequilibration is 30 min.

Optimizing the recovery and resolution of the measurement techniques for individual carotenoids in human serum reduces the possibility of obscured or biased associations in prospective studies of blood carotenoids and cancer. However, carotenoid degradation during storage of blood samples can lead to similar problems. Even with storage under optimal conditions (−70°C), evidence suggests loss of β-carotene (12, 13). Other carotenoids, such as lycopene, may be more labile than β-carotene. At present epidemiologists compensate for degradation by matching noncases to cases on length of storage of serum samples or by standardizing for length of storage in analysis. Nonetheless, more thought should be given to practical ways to protect individual carotenoids from oxidative degradation during long-term storage of biological samples.

Comparison of Food Groups, Indices of Total Carotenoid Intake, and Individual Carotenoid Measures in Cancer Studies

Our third research direction involves the incorporation of the individual carotenoid composition of various foods into analyses of the relationships between diet and cancer in retrospective and prospective studies. Epidemiologic studies have had to rely on the estimates of provitamin A carotenoids in U.S. Department of Agriculture food composition tables (51). These values are not measures of β-carotene or of total carotenoids. They reflect the content of α-carotene, β-carotene, lycopene, cryptoxanthin, and several other chemically similar hydrocarbon carotenoids because the Association of Official Analytical Chemists' approved method for provitamin A carotenoids in foods does not usually resolve these compounds. The U.S. Department of Agriculture and NCI will soon publish a list of the major individual carotenoids in common foods that is based on a scientific evaluation of literature values and new research. These data will be useful in the analysis of published and ongoing epidemiologic studies to refine hypotheses about the role of vegetables, fruits, and carotenoids.

All four retrospective studies of diet and lung cancer comparing the influence of carotenoids and food groups found stronger inverse associations with vegetable and fruit intake than with quantitative estimates of carotenoids (27, 28, 35, 36, 39). One study was a population-based case-control study of incident lung cancer conducted in white men in New Jersey (28); results are shown in Table 7. More pronounced trends in lung cancer risk are seen with vegetables, dark green vegetables, and yellow-orange vegetables than with carotenoids. Similarly, in a population-based case-control study of incident lung cancer conducted in multiethnic men and women in Hawaii (36), total vegetable intake was more predictive of reduced risk in both men and women than was a β-carotene estimate (Table 8). Two explanations of these findings are possible. One is that β-carotene is indeed protective, but food groups rich in β-carotene are better measures of its intake than is an approximate index of the hydrocarbon carotenoids. Alternatively, the protective agent may be another carotenoid or another constituent of vegetables. With more valid measures of the individual carotenoids (including β-carotene) in foods, we hope to distinguish these hypotheses.

In conclusion, epidemiologic studies have demonstrated that

Table 8 *Adjusted relative risks of lung cancer among multiethnic Hawaiians*[a, b]

Nutrient or food group	Level of consumption: Upper 25%	Quartile 3	Quartile 2	Lower 25%	p for trend
Men					
β-Carotene	1.0	1.5	2.4	1.9	0.001
Vegetables	1.0	1.9	2.3	2.7	<0.001
Women					
β-Carotene	1.0	1.9	2.4	2.7	0.01
Vegetables	1.0	3.2	3.0	7.0	<0.001

[a] Included are 230 male cases, 597 male controls, 102 female cases, and 268 female controls.
[b] Adapted from Marchand *et al.* (36).

increased dietary intake of vegetables and fruits and carotenoids and elevated blood levels of β-carotene are consistently associated with reduced risk of lung cancer. Epidemiologic research also suggests that vegetables and fruits and carotenoids may be involved in the etiology of certain other cancers, although fewer studies have been conducted and the results are less consistent. The simplest explanation is that β-carotene is protective, and a number of clinical trials of β-carotene supplements have been initiated to evaluate this specific hypothesis. However, other carotenoids, other constituents of vegetables and fruits, and dietary patterns tightly associated with vegetable and fruit intake need to be explored further as alternatives to the β-carotene hypothesis.

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